

1 WATER-SOLUBLE STABILIZED SELF-ASSEMBLED POLYELECTROLYTES

2
3 FIELD OF THE INVENTION

4 This invention generally relates to the preparation of
5 water-soluble self-assemblies, particularly to the
6 association of ionizable or permanently-charged copolymers,
7 and most particularly to the inclusion of hydrophobic
8 comonomers to the polyelectrolyte segment forming the
9 assembly core.

10
11 BACKGROUND OF THE INVENTION

12 In order to improve the specific delivery of drugs with
13 a low therapeutic index, several drug carriers such as
14 liposomes, microparticles, nano-associates (e.g. polymeric
15 micelles, polyion complex micelles (PICM)) and drug-polymer
16 conjugates have been studied. In recent years, water-soluble
17 supramolecular assemblies such as polymeric micelles and
18 PICM have emerged as promising new colloidal carriers for
19 the delivery of hydrophobic drugs and polyions (e.g.
20 antisense oligonucleotides), respectively.

21 Polymeric micelles have been the object of growing
22 scientific attention, and have emerged as potential carriers

1 for drugs having poor water solubility because they can
2 solubilize those drugs in their inner core and they offer
3 attractive characteristics such as a generally small size
4 (<300nm) and a propensity to evade scavenging by the
5 mononuclear phagocyte system.

6 Micelles are often compared to naturally occurring
7 carriers such as viruses or lipoproteins. All three of
8 these carriers demonstrate a similar core-shell structure
9 that allows for their contents to be protected during
10 transportation to the target cell, whether it is DNA for
11 viruses or water-insoluble drugs for lipoproteins and
12 micelles.

13 Polymeric micelles seem to be one of the most
14 advantageous carriers for the delivery of poorly water-
15 soluble drugs as reported by Jones and Leroux, *Eur. J.*
16 *Pharm. Biopharm.* (1999) 48, 101-111; Kwon and Okano, *Adv.*
17 *Drug Deliv. Rev.* (1996) 21, 107-116 and Allen et al.
18 *Colloids Surf. B: Biointerf.* (1999) 16, 3-27. They are
19 characterized by a core-shell structure. The hydrophobic
20 inner core generally serves as a microenvironment for the
21 solubilization of poorly water-soluble drugs, whereas the
22 hydrophilic outer shell is responsible for micelle

1 stability, protection against opsonization, and uptake by
2 the mononuclear phagocyte system.

3 Pharmaceutical research on polymeric micelles has been
4 mainly focused on copolymers having an AB diblock structure
5 with A, the hydrophilic shell moieties and B the hydrophobic
6 core polymers, respectively. Multiblock copolymers such as
7 poly(ethylene oxide)-poly(propylene oxide)- poly(ethylene
8 oxide) (PEO-PPO-PEO) (A-B-A) can also self-organize into
9 micelles, and have been described as potential drug
10 carriers, e.g. Kabanov et al., *FEBS Lett.* (1989) 258, 343-
11 345. The hydrophobic core which generally consists of a
12 biodegradable polymer such as a poly(β -benzyl-aspartate)
13 (PBLA), poly(D,L-lactic acid) or poly(ϵ -caprolactone), serves
14 as a reservoir for a poorly water-soluble drug, protecting
15 it from contact with the aqueous environment. The core may
16 also consist of a water-soluble polymer, such as
17 poly(aspartic acid) (P(Asp)), which is rendered hydrophobic
18 by the chemical conjugation of a hydrophobic drug, or is
19 formed through the association of two oppositely charged
20 polyions (PICM). Several studies also describe the use of
21 poorly or non-biodegradable polymers, such as polystyrene
22 (PSt) or poly(methyl methacrylate) (PMMA), as constituents of

1 the inner core. See, e.g., Zhao et al., *Langmuir* (1990) 6,
2 514-516; Zhang et al., *Science* (1995) 268, 1728-1731; Inoue
3 et al., *J. Controlled Release* (1998) 51, 221-229 and Kataoka
4 *J. Macromol. Sci. Pure Appl. Chem.* (1994) A31, 1759-1769.
5 The hydrophobic inner core can also consist of a highly
6 hydrophobic small chain such as an alkyl chain or a
7 diacyllipid (e.g. distearoyl phosphatidyl ethanolamine).
8 The hydrophobic chain can be either attached to one end of a
9 polymer, or randomly distributed within the polymeric
10 structure. The shell usually consists of chains of
11 hydrophilic, non-biodegradable, biocompatible polymers such
12 as poly(ethylene oxide) (PEO) (see Allen et al. *Colloids*
13 *Surf. B: Biointerf.* (1999) 16, 3-27 and Kataoka et al. *J.*
14 *Controlled Release* (2000) 64, 143-153), poly(N-vinyl-2-
15 pyrrolidone) (PVP) (see Benahmed A et al. *Pharm Res* (2001)
16 18, 323-328) or poly(2-ethyl-2-oxazoline) (see Lee et al.
17 *Macromolecules* (1999) 32, 1847-1852).

18 The biodistribution of the carrier is mainly dictated
19 by the nature of the hydrophilic shell. Other polymers such
20 as poly(N-isopropylacrylamide) and poly(alkylacrylic acid)
21 impart temperature or pH sensitivity to the micelles, and
22 could eventually be used to confer bioadhesive properties

1 (see US Patent 5,770,627). Micelles presenting functional
2 groups at their surface for conjugation with a targeting
3 moiety have also been described (See, e.g., Scholz, C. et
4 al., *Macromolecules* (1995) 28, 7295-7297).

5 At the present time, most polymeric micelles described
6 in the literature are prepared using non-ionizable block
7 polymers or block copolymers where ionizable monomers are
8 used to form the micelle corona whereas the core consists of
9 a hydrophobic neutral homopolymer or copolymer. Ionizable
10 diblock copolymers have been shown to exhibit pH-dependent
11 micellization. Recently, Webber and Martin (U.S. Patent
12 5,955,509) have described a type of pH-dependent micelles
13 with a polyelectrolyte core. These micelles are composed of
14 the diblock copolymers poly(vinyl N-heterocycle)-*block*-
15 poly(alkylene oxide). Such copolymers are positively charged
16 at acidic pH due to the protonation of the nitrogen atoms,
17 and thus exist as unimers in acidic solutions. At high pH,
18 the unprotonated copolymers self-associate into polymeric
19 micelles. These micelles are primarily intended to deliver
20 their contents at low pH, since the dissociation of the
21 supramolecular assembly under acidic conditions allows a
22 drug to be released. Such conditions may be found, for

1 example in tumors. If intended to be administered by the
2 oral route, these micelles would rapidly release their
3 contents in the stomach because of its acidic pH.
4 Therefore, for oral delivery, they should be formulated
5 using an enteric coating to prevent premature drug leakage.

6 A potential problem with ionizable copolymers is the
7 possibility of forming, at acidic pH, intra and inter-
8 molecular hydrogen bonding between the protonated and the
9 non-ionizable hydrophilic blocks which might lead to the
10 formation of an insoluble complex. This has been recently
11 described by Lele et al. *J. Controlled Release* (2000) 69,
12 237-248, between poly(acrylic acid) and poly(ethylene
13 glycol). Precipitation of the micelles at acidic pH could
14 potentially compromise the efficacy of the system when oral
15 delivery is envisaged.

16 PICM have a block or graft copolymer architecture and
17 consist of a polyelectrolyte linked to a non-ionic water-
18 soluble polymer. They bind with charged compounds due to
19 electrostatic interactions with the polyelectrolyte (see,
20 e.g., Kataoka et al. *Macromolecules* (1996) 29, 8556-8557).
21 The complexes self-assemble into micelle-like structures
22 which have a hydrophobic core from neutralized

1 polyelectrolyte and counterion, and hydrophilic corona.
2 PICM show improved solubility compared with other
3 electrostatic complexes. Furthermore, they show reduced
4 affinity for plasma components and can protect active
5 compounds such as DNA against enzymatic degradation.

6 Although, PICM hold great promise as carriers for a
7 variety of selected from the group consisting of ionizable
8 and permanently-charged diblock copolymers, ionizable and
9 permanently-charged multiblock copolymers, and ionizable and
10 permanently-charged random copolymers with grafted
11 hydrophilic and essentially non-ionic oligomers or polymers
12 compounds, such as charged drugs and nucleic acids, some
13 important issues still remain to be addressed. For instance,
14 the stability of the polymeric micelles is influenced by
15 various factors such as concentration, temperature and
16 chemical structure of the polymer. In particular, the
17 presence of salts is a key parameter for the dissociation of
18 PICM since Coulombic interactions between charged segments
19 are screened by the added salt. To overcome this problem,
20 polymeric micelles can be stabilized by cross-linking the
21 core or shell (see, e.g., Kakizawa et al. *J. Am. Chem. Soc.*
22 (1999) 121, 11247-11248). However, cross-linking the core or

1 shell can potentially chemically alter the active agent
2 and/or excessively slow down its release from the micelles.

3

4 DESCRIPTION OF THE PRIOR ART

5 U.S. patent 5,693,751 teaches the preparation of
6 polymeric micelles composed of a water-soluble block
7 copolymer having a hydrophilic segment and a hydrophobic
8 pharmacological-functioning segment to a side chain of which
9 a drug is covalently bonded.

10 U.S. patent 5,702,717 teaches the preparation of a
11 solution comprising a drug and a block amphiphilic copolymer
12 of poly(ethylene glycol) and poly(α -hydroxy acids) or
13 poly(ethylene carbonates). These copolymers are not
14 polyelectrolytes.

15 U.S. patent 5,939,453 teaches the preparation of
16 polymeric micelles and bioerodible drug delivery matrix
17 using poly(ethylene glycol)-poly(orthoester) diblock and
18 triblock copolymers. The principal novelty of this invention
19 relies on the use of poly(orthoester) as the hydrophobic,
20 bioerodible segment. These block copolymers are neither
21 ionizable or permanently charged.

22 U.S. patent 5,786,387 teaches the preparation of

1 supramolecular assemblies using lipid double chain
2 derivatives containing poly(oxyethylene) for drug delivery
3 applications. These block copolymers are neither ionizable
4 or permanently charged. They efficiently are able to avoid
5 the reticuloendothelial system and possess a long
6 circulation time.

7 U.S. patent 5,840,319 teaches the preparation of
8 polymeric micelles using polyether block copolymers having a
9 critical micelle concentration of no more than 0.5% (w/w) at
10 37°C in an isotonic aqueous solution. The formulation also
11 contains a chemotherapeutic agent.

12 U.S. patent 5,770,627 teaches the preparation of
13 hydrophobically-modified bioadhesive polyelectrolytes. These
14 polyelectrolytes can form micellar structures in aqueous
15 solutions (example 6) and be loaded with an active agent.
16 The polyelectrolyte can be a graft or block copolymer. The
17 outer shell is ionizable since it contains carboxylic acid
18 groups whereas the inner core consists of a homopolymer,
19 copolymer, unsaturated or saturated alkyl chains, or other
20 hydrophobic moities. Methods of administering such agents
21 to an animal are disclosed.

22 U.S. Patent 5,955,509 relates to the use of poly(vinyl

1 N-heterocycle)-block-poly(alkylene oxide) copolymers in
2 micelle containing pharmaceutical formulations. The
3 copolymers advantageously respond to pH differences in the
4 environment to which they are exposed forming micelles at
5 higher pH values. The micelles, which comprise a therapeutic
6 compound and a copolymer, deliver drug in a pH dependent
7 manner.

8 U.S. Patent 5,929,177 provides a block polymer which
9 has functional groups on both ends thereof, and which
10 comprises hydrophilic/hydrophobic segments. As for the
11 functional groups on its both ends, the block polymer has
12 amino group, carboxyl group or mercapto group on the alpha
13 terminal, and hydroxyl group, carboxyl group, aldehyde group
14 or vinyl group on the omega terminal. Hydrophilic segment
15 comprises polyethylene oxide, while hydrophobic segment is
16 derived from lactide, lactone or (meth)acrylic acid ester.
17 The block polymer of this invention forms a polymeric
18 micelle which is usable as bio-compatible materials.

19 U.S. patent 5,925,720 provides a heterotelechelic
20 oligomer or polymer which can be prepared by means of living
21 polymerization and which can form stable micelles in an
22 aqueous solvent. In this invention, there is no reference to

1 stabilization of a polyelectrolyte micelle core.

2 U.S. Patent 5,656,611 relates to compositions for
3 stabilizing polynucleic acids using polyionic complexes. In
4 one aspect the invention provides a polynucleotide complex
5 between a polynucleotide and a block copolymer comprising a
6 polyether block and a polycation block.

7 U.S. patent 6,217,912 provides a biodegradable
8 composition suitable for delivering a gene into a cell.

9 U.S. patent 6,221,959 provides composition for
10 stabilizing polynucleic acids and increasing the ability of
11 polynucleic acid to cross cell membranes and act in the
12 interior of a cell. In one aspect the invention provides a
13 polynucleotide complex between a polynucleotide and certain
14 polyether block copolymer.

15 U.S. patent 5,510,103 relates to drug carriers composed
16 of a block copolymer having hydrophilic and hydrophobic
17 segments, a polymeric type drug comprising hydrophobic drugs
18 trapped by physical treatments in said drug carrier and
19 methods for trapping hydrophobic drugs in the carrier.

20 What is lacking in the art are water-soluble
21 supramolecular assemblies having a physically-stabilized
22 polyelectrolyte core and an uncharged hydrophilic shell and

1 techniques for their preparation.

2

3 SUMMARY OF THE INVENTION

4 The present invention is directed toward water-soluble
5 supramolecular self-assemblies and a process for their
6 preparation via micellization of polyelectrolytes through
7 the use of hydrophobic monomeric units. In this invention
8 the polyelectrolyte segment ultimately forms the core of the
9 supramolecular assembly whereas the shell consists of
10 uncharged hydrophilic polymers or oligomers. It has been
11 determined that the inclusion of the hydrophobic co-monomers
12 to the polyelectrolyte segment forming the micelle core
13 leads to a structure of enhanced stability. Such co-
14 monomers, by increasing the attractive forces between the
15 segments of the core, stabilize the micelles and/or decrease
16 the interaction between the ionizable or permanently-charged
17 segment and non-ionizable segment of the copolymer.

18 Accordingly, it is an objective of the instant
19 invention to provide a stabilized supramolecular assembly
20 and a process for its production.

21 It is a further objective of the instant invention to
22 provide, also through the use of hydrophobic monomers, pH-
23 dependent polymeric micelles or polyion complexes which

1 exhibit reduced interaction between the ionizable or
2 permanently-charged segment and the non ionizable segments
3 of the copolymer outer shell.

4 It is yet another objective of the instant invention to
5 provide a pH dependent micellar vehicle, suitable as a
6 carrier for pharmacological constituents which is not
7 subject to the untoward formation of insoluble complexes.

8 It is a still further objective of the invention to
9 teach a process for preparing stabilized supramolecular
10 assemblies having a polyelectrolyte core, through the use of
11 hydrophobic monomeric units.

12 Other objects and advantages of this invention will
13 become apparent from the following description taken in
14 conjunction with the accompanying drawings wherein are set
15 forth, by way of illustration and example, certain
16 embodiments of this invention. The drawings constitute a
17 part of this specification and include exemplary embodiments
18 of the present invention and illustrate various objects and
19 features thereof.

20

21 BRIEF DESCRIPTION OF THE FIGURES

22 Figure 1 is a synthetic route for the preparation of block

1 copolymer, possessing a hydrophobized polyelectrolyte block;
2 Figure 2 is the ^1H NMR spectrum of PEG-*b*-P(DMAEMA₇₀-co-EMA₃₀)
3 in CDCl_3 ;
4 Figure 3 shows the variation in light scattering and pyrene
5 fluorescence I_{338}/I_{333} ratio of a PEG-*b*-P(EA₅₀-co-MAA₅₀) aqueous
6 solution as a function of pH;
7 Figure 4 shows the ^1H NMR spectrum of PEG-*b*-P(EA₅₀-co-MAA₅₀)
8 in D_2O at pH 10 (A) and at pH 3 (B).

9 10 DETAILED DESCRIPTION OF THE INVENTION

11 In the present invention, the terms "water-soluble
12 self-assemblies" and "micelles" are equally employed
13 although the proposed structures may not necessarily
14 correspond to the true definition of micelles.

15 Micelle formation occurs as a result of two forces.
16 One is an attractive force that leads to the association of
17 molecules, while the other is a repulsive force that
18 prevents unlimited growth of the micelles to a distinct
19 macroscopic phase. Amphiphilic copolymers self-associate
20 when placed in a solvent that is selective for either the
21 hydrophilic or hydrophobic polymer.

22 The micellization process of amphiphilic copolymers is

1 similar to that for low molecular weight surfactants. At
2 very low concentrations, the polymers exist only as single
3 chains. As the concentration increases to reach a critical
4 value called the critical association concentration ("CAC"),
5 polymer chains start to associate to form micelles in such a
6 way that the hydrophobic part of the copolymer will avoid
7 contact with the aqueous media in which the polymer is
8 diluted. Amphiphilic copolymers usually exhibit a CAC which
9 is much lower than that of low molecular weight surfactants.
10 For example, the CAC of PEO-PBLA and PNIPA-PSt are between
11 0.0005-0.002%. Some amphiphilic copolymers, however,
12 exhibit much higher CAC, reaching up to 0.01-10% in the case
13 of poloxamers. Amphiphilic copolymers with high CAC may not
14 be suitable as drug targeting devices since they are
15 unstable in an aqueous environment and are easily
16 dissociated upon dilution.

17 Micelles can be targeted to specific cells or
18 tissues via the inclusion of targeting ligands, e.g.
19 monoclonal antibodies, lectins, sugars, vitamins, peptides
20 or immunologically distinct fragments thereof or the like
21 moieties which provide the micelles with an ability to
22 preferentially concentrate in a particular target area.

1 The micellization of amphiphilic copolymers can result
2 in two different types of micelles depending on whether the
3 hydrophobic chain is randomly bound to the hydrophilic
4 polymer or grafted to one end of the hydrophilic chain.
5 Micelles formed from randomly modified polymers are
6 generally smaller than end-modified polymers. The micellar
7 size is mainly determined by the hydrophobic forces which
8 sequester the hydrophobic chains in the core, and by the
9 excluded volume repulsion between the chains which limits
10 their size. The difference in the balance of these two
11 forces in random and end-modified copolymers may account for
12 their different size.

13 Determination of Critical Association Concentration (CAC):

14 Light scattering is widely used for the determination
15 of the molecular weight and aggregation number of micelles.
16 The onset of micellization can, however, be detected only if
17 the CAC falls within the sensitivity of the scattering
18 method. This is rarely the case for polymers in water. Gel
19 permeation chromatography (GPC) under aqueous conditions can
20 be employed since single chains and micellar fractions of
21 copolymers exhibit different elution volumes. It is also
22 possible to simultaneously determine by GPC the molecular

1 weight of the micelles and their aggregation number.

2 A preferred method to determine the CAC involves the
3 use of fluorescent probes, among which pyrene is widely
4 used. Pyrene is a condensed aromatic hydrocarbon that is
5 highly hydrophobic and sensitive to the polarity of the
6 surrounding environment. Below the CAC, pyrene is
7 solubilized in water, a medium of high polarity. When
8 micelles are formed, pyrene partitions preferentially toward
9 the hydrophobic domain afforded by the micellar core, and
10 thus experiences a nonpolar environment. Consequently,
11 numerous changes such as an increase in the fluorescence
12 intensity, a change in the vibrational fine structure of the
13 emission spectra, and a red shift of the (0,0) band in the
14 excitation spectra are observed. The apparent CAC can be
15 obtained from the plot of the fluorescence of pyrene, the
16 I_1/I_3 ratio from emission spectra or the I_{338}/I_{333} ratio from
17 the excitation spectra versus concentration. A major change
18 in the slope indicates the onset of micellization. Changes
19 in anisotropy of fluorescent probes have also been
20 associated with the onset of micellization. E.g. see Jones
21 and Leroux *Eur. J. Pharm. Biopharm.* (1999) 48, 101-111.

22 Polymeric micelles such as those of the compositions of

1 the invention are characterized by their small size.
2 Besides being needed for extravasation of the carrier
3 materials, this small size permits the sterilization of the
4 composition to be effected simply by filtration, and
5 minimizes the risks of embolism in capillaries after
6 intravenous injection. Micellar size depends on several
7 factors including copolymer molecular weight, relative
8 proportion of hydrophilic and hydrophobic chains and
9 aggregation number.

10 Micellar diameter and size polydispersity can be
11 obtained directly in water or in an isotonic buffer by
12 dynamic light scattering (DLS). Micelle size can also be
13 estimated by methods such as atomic force microscopy (AFM),
14 transmission electron microscopy (TEM) and scanning electron
15 microscopy (SEM). These methods allow the characterization
16 of the micelle shape and size dispersity.
17 Ultracentrifugation velocity studies are sometimes performed
18 to assess the polydispersity of polymeric micelles.

19 Loading of one or more pharmacological constituents,
20 e.g. various therapeutic agents, drugs, peptides, proteins,
21 genetic material (e.g. oligonucleotides), genetically
22 altered constituents, polyionic constituents and the like,

1 into the micelles can be realized according to techniques
2 well known to one skilled in the art. For example, drugs
3 can be incorporated into the polymeric micelle compositions
4 of the invention by means of chemical conjugation or by
5 physical entrapment through dialysis, emulsification
6 techniques, simple equilibration of the drug and micelles in
7 an aqueous medium or solubilization of a drug/polymer solid
8 dispersion in water.

9 Therapeutic agents which may be used are any compounds,
10 including the ones listed below, which can be entrapped, in
11 a stable manner, in polymeric micelles and administered at a
12 therapeutically effective dose. Preferably, the therapeutic
13 agents used in accordance with the invention are hydrophobic
14 or polyionic (e.g. DNA). Suitable drugs include antitumor
15 compounds such as phthalocyanines (e.g. aluminum chloride
16 phthalocyanine), anthracyclines (e.g. doxorubicin), poorly
17 soluble antimetabolites (e.g. methotrexate, mitomycin, 5-
18 fluorouracil) and alkylating agents (e.g. carmustine).
19 Micelles may also contain taxanes such as paclitaxel.

20 Additional drugs which can be contained in micelles are
21 conventional hydrophobic antibiotics and antifungal agents
22 such as amphotericin B and itraconazole, poorly water-

1 soluble immunomodulators such as cyclosporin, poorly water-
2 soluble antiviral drugs such as HIV protease inhibitors and
3 poorly water-soluble steroidal (e.g. dexamethasone), and
4 non-steroidal (e.g. indomethacin) anti-inflammatory drugs.

5 Hydrophilic compounds such as proteins may also be
6 incorporated in the polymeric micelle compositions of the
7 invention. The incorporation of such hydrophilic species
8 may, however, require the chemical hydrophobization of the
9 molecule or a particular affinity for the hydrophilic shell.
10 Polyionic compounds (e.g. antisense oligonucleotides, genome
11 fragments, peptides) can be incorporated into micelles
12 through the formation of PICM via electrostatic interaction
13 with an oppositely charged block polyelectrolyte.

14 The polymeric micelle compositions of the invention are
15 suitable for use in a variety of pharmaceutical fields, such
16 as oral delivery, sustained release and site-specific drug
17 targeting. Preferably, the micelles of the invention are
18 used as a transport for water-insoluble and polyionic
19 compounds. Included within the scope of the invention are
20 supramolecular assembly compositions comprising a suitable
21 targeting ligand.

1 Without intending to be limited to a particular
2 synthesis procedure, block polyelectrolytes useful in the
3 present invention are most preferably prepared by
4 "living"/controlled radical polymerization (LCRP), such as
5 atom transfer radical polymerization (ATRP) (see Coessens et
6 al., *Prog. Polym. Sci.* (2001) 26, 337-377) or nitroxide-
7 mediated radical polymerization (NMP) (see Benoit et al. *J.*
8 *Am. Chem. Soc.* (1999), 121, 3904-3920). However, any
9 alternative procedure such as other living radical
10 polymerizations or condensation of preformed functionalized
11 polymers could also be used. On the other hand, (i)
12 ionizable and permanently-charged multiblock copolymers,
13 (ii) ionizable and permanently-charged amphiphilic random
14 copolymers with grafted hydrophilic oligomers (or polymers)
15 could be used instead of block copolymers for diverse
16 applications within the scope of the presently disclosed
17 invention.

18 The radical initiator for the synthesis of the polymer
19 by ATRP can be any appropriately functionalized molecule
20 (e.g. poly(ethylene glycol) (PEG), PVP). The initiator bears
21 an halogeno functionality that can be activated for ATRP
22 (see Coessens et al., *Prog. Polym. Sci.* (2001) 26, 337-377).

1 Without intending to be limited to any particular
2 substituent, this functionality can be a 2-
3 halogenoisobutyrylate derivative, 2-halogenopropionate
4 derivative, 2-halogenoacetate derivative or 1-
5 (halogenomethyl)benzene derivative. For other types of
6 polymerizations (e.g. NMP), this functionality is
7 appropriately chosen according to the used monomers.

8 The catalyst for the ATRP usually includes a metallic
9 salt and a ligand. The ligand is used for the
10 solubilization of the salt in organic solvent and/or to
11 activate the redox reaction of the metal present in the
12 salt. The salt activates the radical initiator for the ATRP.
13 Without intending to be limited to any particular salt, the
14 latter can be copper(I) bromide, copper(I) chloride or
15 copper(I) thiocyanate, iron(II) and nickel(0 or I)
16 compounds. The ligand can include 2,2'-bipyridine
17 derivatives or bis(dimethylamino) compounds (e.g.
18 N,N,N',N',N'',N''-pentamethyldiethylene-triamine (PMDETA).

19 Suitable polyelectrolyte compounds useful in the
20 preparation of supramolecular self-assemblies may be
21 selected from the group consisting of diblock copolymers
22 including ionizable units, permanently charged units or

1 mixtures of ionizable and permanently charged units,
2 multiblock copolymers including ionizable units, permanently
3 charged units or mixtures of ionizable and permanently
4 charged units, and random copolymers with grafted
5 hydrophilic and essentially non-ionic oligomers or polymers,
6 said random copolymers including ionizable units,
7 permanently charged units or mixtures of ionizable and
8 permanently charged units.

9 The diblock copolymers generally consist of two blocks,
10 one of which is hydrophilic and generally uncharged and the
11 other containing at least one compound selected from the
12 group consisting of ionizable and permanently-charged
13 repeating units (or combinations thereof) in combination
14 with essentially hydrophobic, e.g. hydrophobic or relatively
15 hydrophobic non-ionic monomers. Ionizable units refers to
16 repeating units that can be transformed from a non-ionic to
17 a charged state via an external stimulus (e.g. pH or
18 chemical reaction). Permanently charged units refers to
19 repeating units that are in fact electrostatically charged
20 irrespective of the external conditions.

21 The ionizable and/or permanently-charged block, bearing
22 hydrophobic repeating units, can be synthesized from vinyl

1 monomers, vinyl oligomers or eventually vinyl polymers.
2 These hydrophobic monomers/oligomers/polymers can be
3 acrylate, acrylamide, alkylacrylate, alkylacrylamide,
4 arylacrylate and arylacrylamide derivatives for which the
5 alkyl and aryl term stands for aliphatic or aromatic
6 moieties respectively (e.g. methacrylate, methacrylamide
7 derivatives.) The hydrophobic compound can also be a
8 biodegradable polyester such as vinyl-terminated
9 poly(lactide) and vinyl-terminated poly(ϵ -caprolactone). The
10 ionizable monomers could be alkylacrylic acid derivatives,
11 (aminoalkyl)acrylate or (aminoalkyl)alkylacrylate
12 derivatives. The acidic or basic units of the polymer chain
13 can be derived from a non-ionizable precursor (e.g. tert-
14 butylmethacrylate), which is cleaved into an acidic moiety.
15 The hydrophilic block can be synthesized from vinyl
16 monomers, vinyl oligomers or eventually vinyl polymers.
17 These hydrophilic monomers/oligomers/polymers can be
18 acrylate, acrylamide, alkylacrylate and alkylacrylamide
19 (e.g. PEG methacrylate and N-(2-hydroxypropyl)acrylamide).
20 On the other hand, the hydrophilic block can also originate
21 from a block radical macroinitiator based on PEG or PVP
22 derivatives.

1 Non biodegradable ionizable and/or permanently-charged
2 copolymers that are intended to be administered
3 parenterally, should have a molecular weights not exceeding
4 40,000. There is no restriction on molecular weights for
5 biodegradable or non-biodegradable ionizable copolymers,
6 which are used orally or locally.

7 The loading of poorly water-soluble and non-ionic drugs
8 should be done in an organic solvent, or in aqueous
9 solutions at a pH where the core is uncharged or mostly
10 uncharged. Charged drugs should be loaded under conditions
11 (e.g. pH) where electrostatic interactions with the
12 ionizable or permanently-charged segment are possible.

13
14 Examples

15 Abbreviations:

16 The subscript text indicates the ratio in a polymeric
17 segment. The letter b features that polymers and/or
18 polymeric arms are based on a diblock copolymeric structure.
19 The term co means the repeating units are disposed randomly
20 along the polymeric segment.

21

1 Example 1

2 Synthesis of poly(ethylene glycol)-block-poly(N,N-
3 dimethylaminoethanemethacrylate-co-ethylmethacrylate) with a
4 ratio for DMAEMA/EMA of 70/30.

5 PEG-b-P(DMAEMA₇₀-co-EMA₃₀)

6

7 Materials:

8 All products were purchased from Aldrich (Milwaukee,
9 WI). Copper(I) bromide (99.99% Grade), 2-bromoisobutyryl
10 bromide, anhydrous triethylamine and N,N,N',N',N'',N''-
11 pentamethyldiethylenetriamine (PMDETA) were used without
12 further purification. Poly(ethylene glycol) monomethyl ether
13 (MeO-PEG-OH, M_n: 2000) was dried with toluene by an
14 azeotropic distillation before use. Ethyl methacrylate (EMA)
15 and 2-(N,N-dimethylamino)ethyl methacrylate (DMAEMA) were
16 used as vinyl monomers and distilled before polymerization.
17 Prior to use, tetrahydrofuran (THF) was distilled over
18 sodium, using benzophenone as drying indicator.

19

20 Synthesis of PEG ATRP macroinitiator (α -(2-
21 bromoisobutyrylate)- ω -methylPEG):

1 As exemplified in Figure 1, to a solution of MeO-PEG-OH
2 (M_n : 2000, 10 g, 0.005 mol) and triethylamine (1.0 g, 0.01
3 mol) in 70 mL of anhydrous THF, slightly cooled in a water-
4 ice bath, was slowly added 2-bromoisobutyryl bromide (4.3
5 mL, 0.035 mol). The solution was then warmed to room
6 temperature and stirred for 24 h. The mixture was poured
7 into water and extracted with methylene chloride. The
8 organic extracts were washed successively with a HCl 1M and
9 NaOH 1M solution (containing NaCl), and dried over magnesium
10 sulfate. The solvent was removed under reduced pressure. The
11 crude was dissolved in a minimum of methylene chloride, and
12 then precipitated in diethyl ether. The title compound was
13 recovered by simple filtration. Yield: 70% after
14 precipitation. White solid. M.P. 60-65°C.
15 ^1H NMR (δ , ppm, CDCl_3): 4.18 (2H); 3.50 (188H); 3.23 (3H, s);
16 1.80 (6H, s).

17
18 ATRP:

19 The ATRP of monomers was carried out in bulk and in
20 solution, using α -(2-bromoisobutyrylate)- ω -methyl-PEG as
21 ATRP macroinitiator. The PEG ATRP macroinitiator (1 eq.) was
22 added to a solution containing PMDETA (1.1 eq.), Cu(I)Br

1 (1.1 eq.), EMA (6 eq) and DMAEMA (14 eq) in THF (0.8 M). The
2 mixture was degassed with argon for 15-20 min at room
3 temperature and was then heated to 60°C overnight. After the
4 polymerization, the mixture was poured in THF, containing 10
5 % of methanol. The resulting polymers were filtered on
6 silica gel, with THF as eluent, to remove copper bromide.
7 Finally, polymers were dialyzed (SPECTRA/POR No.1, molecular
8 weight cutoff 6000-8000) against water during 48 h and then
9 freeze-dried. Yield: 98%. (Figure 1)

10

11 Poly(ethylene glycol)-*block*-poly(N,N-
12 dimethylaminoethanemethacrylate-co-ethylmethacrylate).

13 PEG-*b*-P(DMAEMA₇₀-co-EMA₃₀)

14 ¹H NMR (δ, ppm, MeOD): 4.30 (18H); 4.04 (32H); 3.60 (182H);
15 3.38 (3H); 2.69 (54H), 2.05-1.87 (42H); 1.43 (6H); 1.26
16 (56H); 1.05 and 0.88 (73H).

17

18 Polymer and micelle characterization:

19 ¹H and ¹³C NMR spectra were recorded on a Bruker AMX300
20 and ARX400 in deuterated chloroform (CDCl₃) and methanol
21 (CD₃OD) (CDN Isotopes, Canada) at 25°C. Number- (M_n) and
22 weight-average (M_w) molecular weights were determined by size

1 exclusion chromatography (SEC) with an Alliance GPCV2000
2 (Waters, Milford, MA) and by nuclear magnetic resonance
3 spectroscopy ($^1\text{H-NMR}$). Particle sizes were evaluated by
4 dynamic light-scattering. The apparent CAC was measured by a
5 steady-state pyrene fluorescence method.

6

7 Results:

8 Copper (I) bromide-pentamethyldiethylenetriamine (CuBr-
9 PMDETA) was used as catalyst and gave yields of
10 polymerization approaching 100 % in THF. The reactivities of
11 EMA and DMAEMA in THF were similar, with a k^{app} of 1.95×10^{-4}
12 $\text{Lmol}^{-1}\text{s}^{-1}$. All monomers were completely consumed after 5 h and
13 the M_n obtained experimentally were close to the theoretical
14 values (Table 1). Moreover, the polydispersity index (PI)
15 was approximately 1.4 and corresponded approximately to the
16 polydispersity of PEG macroinitiator used for the
17 preparation of PEG-*b*-P(DMAEMA₃₀-co-EMA₇₀).

18

Table 1.

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M_n and M_w of P(DMAEMA₃₀-co-EMA₇₀).

Copolymers	M_n Theo	M_n NMR	M_n SEC	PI
PEG- <i>b</i> -P(DMAEMA ₃₀ -co-EMA ₇₀)	4690	4900	4700	1.4

1 Figure 2 shows the ^1H NMR spectrum of PEG-*b*-P(DMAEMA₃₀-
2 co-EMA₇₀). The terminal methoxy group of PEG (signal 1, 3.35
3 ppm), was used as a reference to calibrate the integration
4 of the other signals. The integration of signal 2 showed
5 that the degree of polymerization (DP) of ethylene oxide
6 (PEG chains) was approximately 45 in each copolymer and
7 corresponded to the DP of the commercial PEG used. The
8 narrow signal of the PEG ATRP macroinitiator at 1.80 ppm,
9 assigned to the methyl of the bromoisobutyryl group,
10 decreased rapidly at the beginning of the polymerization and
11 shifted within 10 min to about 1.4 ppm (signal 3),
12 confirming that all PEG chains were coupled to the polyvinyl
13 segments. The signals assigned to the methylene groups in
14 the backbone were observed at 1.8 ppm (signals 4 and 4') and
15 are represented by 3 successive peaks of decreasing
16 intensity. The polymethacrylate derivatives presented two
17 signals assigned to the methyl attached to the backbone
18 (signals 5 and 5'). The ratios were determined using the
19 signals 7, 8 and 9.

20 The CAC of PEG-*b*-P(DMAEMA₃₀-co-EMA₇₀) was determined in
21 water and phosphate buffered saline (PBS, pH 7.4) to verify
22 whether salts could interfere with self-assembling. A

1 remarkable fact was that the PEG-*b*-P(DMAEMA₃₀-co-EMA₇₀) showed
 2 a low CAC in water and PBS, despite the presence of DMAEMA
 3 which is ionized in these aqueous solutions (Table 2). This
 4 could be explained by the presence of EMA in the polymer.
 5 Interestingly, the CAC was not significantly affected by the
 6 presence of salts in water. This is an important issue,
 7 since a common drawback about PICM is their relative poor
 8 stability in physiological media. Accordingly, the
 9 stability of PICM can be easily increased by introducing a
 10 hydrophobic comonomer in the polymer backbone. With regard
 11 to the micelle sizes for PEG-*b*-P(DMAEMA₃₀-co-EMA₇₀) the nature
 12 of the aqueous solution (water vs PBS) seemed to influence
 13 the proportion of secondary aggregates (Table 2).

14 **Table 2**

15 Micellar properties of PEG-*b*-P(DMAEMA₃₀-co-EMA₇₀)

Copolymer	DP of the polyvinyl block	CAC (mg/L) ±10%	Micelle size (nm) SD ±25%	Size Peak amount
PEG- <i>b</i> -P(DMAEMA ₃₀ -co-EMA ₇₀) In water	20	2	392 22	79% 21%
PEG- <i>b</i> -P(DMAEMA ₃₀ -co-EMA ₇₀) In PBS	20	3	280 79	24% 76%

1 Example 2

2 Synthesis of a diblock copolymer containing methacrylic acid
3 units as ionizable units:

4 PEG-*b*-P(EA₅₀-CO-tBMA₅₀) (precursor)

5 PEG-*b*-P(EA₅₀-CO-MAA₅₀)

6
7 Materials, synthesis of PEG-ATRP macroinitiator and ATRP:

8 Carried out as described in Example 1. However, the
9 only difference was 5 eq. of EMA and 5 eq. of tert-butyl
10 methacrylate (tBMA) versus 1 eq. of PEG-ATRP macroinitiator
11 were used for the polymerization of PEG-*b*-P(EA₅₀-CO-tBMA₅₀).
12 In the case of PEG-*b*-P(MAA), only the tBMA monomer (18 eq.)
13 was used. (Figure 1)

14 Transformation of tBMA into MAA:

15 The ester groups, bearing a tert-butyl chain (tBMA),
16 were transformed into carboxylic acid groups, by the
17 cleavage of tert-butyl in acidic conditions. To a solution
18 of the polymers having tBMA units (7.7 mmol) in dioxane (2.6
19 M) was added concentrated HCl (32 mmol) for 5 h. The
20 methacrylic acid derivatives were precipitated in diethyl
21 ether and filtered. The polymers were dissolved in ethanol,
22 dialyzed against water and freeze-dried.

23

1 Polymer and micelle characterization:

2 ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX300
3 and ARX400 in CDCl_3 , in CD_3OD and in D_2O (CDN Isotopes) at
4 different pH, using very small amounts of HCl and NaOH. M_n
5 and M_w were determined by SEC with an Alliance GPVC2000
6 (Waters, Milford, MA) and by NMR spectroscopy. Hydrodynamic
7 mean diameter and size distribution were determined at a 90°
8 angle by DLS using differential size distribution processor
9 intensity analysis (N4Plus, Coulter Electronics, Miami, FL).
10 The apparent CAC was measured by a steady-state pyrene
11 fluorescence method. The pH of self-association was
12 determined by static light scattering at 480 nm, using a
13 Series 2 Aminco fluorimeter. The pH of association was also
14 determined by the steady-state pyrene fluorescence method.
15 Poly(ethylene glycol)-*block*-poly(*tert*-butyl methacrylate)
16 PEG-*b*-P(*t*BMA); M_n : 4560; Using *t*BMA as monomer:

17 ^1H NMR (δ , ppm, CDCl_3): 4.10 (36H); 3.64 (181H); 3.37 (3H, s);
18 2.02-1.80 (36H); 1.44 (6H, s); 1.40 (162H, s); 1.13 (18H,
19 s); 1.02 (36H, s)

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21

22

1 Poly(ethylene glycol)-block-poly(methacrylic acid)
2 PEG-b-P(MAA); M_n : 3180; Obtained from PEG-b-P(tBMA) after the
3 cleavage of tert-butyl groups:

4 ^1H NMR (δ , ppm, MeOD) : 4.19 (36H); 3.66 (186H); 3.38
5 (3H); 2.02 (24H); 1.57 (2H); 1.16 (43H).

6 Poly(ethylene glycol)-block-poly(ethyl acrylate-co-tert-
7 butyl methacrylate); PEG-b-P(EA₅₀-co-tBMA₅₀); M_n : 3600; Using
8 tBMA and EMA as monomers.

9 ^1H NMR (δ , ppm, CDCl₃): 4.10 (12H); 3.66 (181H); 3.39
10 (3H); 2.10-1.70 (22H); 1.44 (54H); 1.28 (18H); 1.00 (22H).

11
12 Poly(ethylene glycol)-block-poly(ethyl acrylate-co-
13 methacrylic acid); PEG-b-P(EA₅₀-co-MAA₅₀); M_n : 3400; Obtained
14 from PEG-b-P(EMA₅₀-co-tBMA₅₀) after the cleavage of tert-butyl
15 groups:

16 ^1H NMR (δ , ppm, MeOD): 4.10 (12H); 3.63 (181H); 3.36
17 (3H); 2.20-1.70 (22H); 1.65 (6H); 1.26 (18H); 1.14 (22H).

18

19 Results:

20 Well-defined acidic (ionizable) copolymers, containing
21 or not hydrophobic units were prepared by ATRP using a
22 PEGylated ATRP macroinitiator. This macroinitiator was

1 synthesized by coupling PEG monomethyl ether to 2-
 2 bromoisobutyryl bromide with a high yield. Copper (I)
 3 bromide-pentamethyldiethylenetriamine (CuBr-PMDETA) was used
 4 as catalyst and gave yields of polymerization approaching
 5 100 % in THF. The M_n estimated by NMR were calculated from
 6 the terminal methoxy group of the PEG chain (~3.4 ppm). All
 7 monomers were completely consumed after 5 h and the M_n
 8 obtained experimentally were close to the theoretical
 9 values. For all copolymers, the polydispersity was within
 10 the range of about 1.3 to about 1.5 (Table 3).

11
 12 **Table 3**

13 Molecular weights of precursors of P(MAA) diblock copolymer derivatives

Copolymer	M_n Theo	M_n NMR	M_n SEC relative	M_n SEC universal	M_w/M_n
PEG ATRP macroinitiator	2150	2250	2100	2200	1.3
PEG- <i>b</i> -P(<i>t</i> BMA)	4460	4560	3600	3900	1.5
PEG- <i>b</i> -P(EA ₅₀ - <i>co</i> - <i>t</i> BMA ₅₀)	3360	3600	3700	4000	1.4

14
 15 The PEG-*b*-P(EA₅₀-*co*-*t*BMA₅₀) and PEG-*b*-P(*t*BMA) precursors
 16 were transformed into their respective P(MAA) derivatives by
 17 cleaving the *tert*-butyl groups in presence of hydrochloric
 18 acid in dioxane. The monomer ratios of the prepared

1 copolyvinyl polymers corresponded approximately to the
2 proportions of monomers used for the polymerization. The
3 hydrophobic unit EA was incorporated in the polyvinyl
4 segment, to increase hydrophobicity. At high pH values, the
5 copolymers are fully ionized and should be in solution
6 mostly as individual polymeric chains. As the pH is
7 decreased, the protonation of the carboxylic groups should
8 increase the hydrophobic character of the copolymer and
9 induce chain aggregation. The pH of interchain association
10 was determined at body temperature (37°C) by static light
11 scattering and by spectrofluorimetry, using pyrene as a
12 probe (Table 2). Figure 3 shows the ratio of fluorescence
13 intensities (I_{338}/I_{333}) versus pH, and the first-order
14 derivative of scattered light as a function of pH for PEG-*b*-
15 P(EA₅₀-co-MAA₅₀). The polymeric chains associated at a pH
16 value close to 6, as determined by both techniques (Figure
17 3).

18 Interestingly, PEG-*b*-P(EA₅₀-co-MAA₅₀) remained soluble at
19 acidic pH, indicating that the protonation of MAA units did
20 not make the diblock copolymers precipitate. In the case of
21 PEG-*b*-P(MAA), a diffuse precipitate appeared when the pH was
22 decreased to around pH 3.5-4. It is known that acrylic acid

1 polymeric derivatives interact through hydrogen bonding with
2 PEG in acidic solutions, resulting in precipitation of the
3 polymers. Without intending to be bound to any specific
4 mechanism, we believe that in the presence of the
5 hydrophobic ethyl acrylate comonomer the attractive forces
6 between the protonated MAA and ethylene oxide units are
7 sterically hindered. Accordingly, since PEG-*b*-P(EA₅₀-co-MAA₅₀)
8 remains soluble at acidic pHs, it is possible that the MAA
9 units become sequestered in the inner core of a
10 supramolecular assembly (possibly micelles) which is
11 stabilized by the PEG chains.

12 To confirm the pH-dependent associative behavior of PEG-
13 *b*-P(EA-co-MAA), its ¹H NMR spectra were recorded in D₂O at pH
14 10 and 3 (Figure 4). At pH 10 (Figure 4A), the copolymer
15 demonstrated all peaks proper to the PEG and the ionized
16 parts, presenting integrations in accordance with the
17 molecular weight (M_n). This suggests that all polymer chains
18 were isolated from each other in water at high pH. However,
19 at low pH (~3), the decreased peaks assigned to P(EA-co-MAA)
20 segments indicated the presence of chain aggregation (Figure
21 4B). Chain aggregation (into polymeric micelles) leads to
22 the formation of a highly viscous internal core and, thus,

1 to a partial suppression of the signals of EA and MAA units.
2 Supporting these results, analysis by DLS revealed, at pH 3,
3 the presence of a colloid population (215 \pm 50 nm), that was
4 absent at pH 10.

5 **Table 4**

6 Determination of the aggregation pH of polymethacrylic acid derivatives

Copolymer	Aggregation pH determined by fluorimetry	Aggregation pH determined by light scattering	Number of MAA per chains*
PEG- <i>b</i> -P (MAA)	5.6	4.9	18
PEG- <i>b</i> -P (EA ₅₀ - <i>co</i> -MAA ₅₀)	5.8	6.0	5

7
8 * Number of MAA units in the polyvinyl segment, evaluated by ¹H NMR
9 spectroscopy from the corresponding copolymers having *tert*-butyl groups.

10

11 All patents and publications mentioned in this
12 specification are indicative of the levels of those skilled
13 in the art to which the invention pertains. All patents and
14 publications are herein incorporated by reference to the
15 same extent as if each individual publication was
16 specifically and individually indicated to be incorporated
17 by reference.

18 It is to be understood that while a certain form of the

1 invention is illustrated, it is not to be limited to the
2 specific form or arrangement of parts herein described and
3 shown. It will be apparent to those skilled in the art that
4 various changes may be made without departing from the scope
5 of the invention and the invention is not to be considered
6 limited to what is shown and described in the specification
7 and drawings.

8 One skilled in the art will readily appreciate that the
9 present invention is well adapted to carry out the objects
10 and obtain the ends and advantages mentioned, as well as
11 those inherent therein. The compounds, compositions,
12 biologically related compounds, methods, procedures and
13 techniques described herein are presently representative of
14 the preferred embodiments, are intended to be exemplary and
15 are not intended as limitations on the scope. Changes
16 therein and other uses will occur to those skilled in the
17 art which are encompassed within the spirit of the invention
18 and are defined by the scope of the appended claims.
19 Although the invention has been described in connection with
20 specific preferred embodiments, it should be understood that
21 the invention as claimed should not be unduly limited to
22 such specific embodiments. Indeed, various modifications of

1 the described modes for carrying out the invention which are
2 obvious to those skilled in the art are intended to be
3 within the scope of the following claims.

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